REMARKS/ARGUMENTS

Claims 1-8 were pending and claims 9-13 have been added. Support for claims 9-13 can be found, for example, in the specification at page 5, lines 16-23; the paragraph bridging pages 8 and 9; and page 3, lines 29-35. Applicant respectfully submits that the present application overcomes all prior rejections and has been placed in condition for allowance for the reasons set forth in detail below.

I. The Invention

The presently claimed invention is directed to a plasmid containing a tightly regulated promoter operatively linked to an isolated and purified DNA sequence that encodes a peptidoglycan-associated lipoprotein (recombinant PAL) of gram-negative bacteria. Under the control of the tightly regulated promoter, the recombinant PAL is expressed in lipidated form and in yields that are higher than those expressed when the recombinant PAL is under the control of a leaky regulated promoter.

II. The Rejection Under 35 USC § 112, Second Paragraph

Claims 1-8 remain rejected as being indefinite for reasons of record, and also because the Examiner alleges that the metes and bounds of "tightly regulated" is now complicated by being compared to an unknown value. The Examiner further alleges that the term "the recombinant PAL" lacks antecedent basis in claim 1. (Office Action, pages 3-4).

Responsive to this rejection, Applicant previously provided the declaration of Dr. Susan Hoiseth, which defines tight, leaky and strong promoters as those terms are understood by a person of at least ordinary skill in the art (see Amendment dated May 13, 2004). Applicant has amended claim 1 in this Amendment and encloses herein a Rule 132 declaration of Dr. Bruce A. Green, Research Fellow of Wyeth Vaccines. Dr. Green provides the results of an experiment designed to examine the relative expression level of lipidated P6 protein from arabinose- and *lac*-promoted plasmid constructs in the same *E. coli* strain.

As stated in Paragraph 6 of the Green Declaration, *Escherichia* coli strain BLR was used as the host strain for expression analysis. Purified plasmid pPX162 (which

contains the gene encoding the lipidated P6 protein under control of the *lac* promoter on a high copy number plasmid) was transformed into *E. coli* BLR and transformants selected on Hy-Soy agar plates containing carbenicillin (carb;100 μg/ml). *E. coli* BLR(pPX4020) (which contains the gene encoding the lipidated P6 protein under control of the arabinose promoter) was also used in these experiments and selected using chloramphenicol (cam; 30 μg/ml).

As stated in Paragraph 7 of the Green declaration, overnight cultures of each bacterial strain grown in liquid HySoy medium containing either carb or cam were diluted 1:40 in HySoy containing the appropriate antibiotic and grown to an OD_{600} ~1.0. Strains BLR(pPX4020) and BLR(pPX162) were induced with 0.2% arabinose and 1mM IPTG, respectively, and grown for three additional hours. Whole cell lysates in SDS-PAGE cracking buffer containing β-mercaptoethanol were made by boiling at ~100 °C for 10 minutes. Lysate equivalent to approximately 10^7 cfu were loaded onto each lane of a 4-20% Tris-Glycine gel and electrophoresed in SDS-PAGE buffer. Gels were stained with Coomassie brilliant blue using standard methods. For Western blot analysis, mAb G125-6TX, which is specific for the *Haemophilus influenzae* P6 protein, was used at 1:5000 followed by goat α-mouse Ig-biotin @ 1:3000.

As stated in Paragraph 8 of the Green declaration, SDS-PAGE analysis was used for evaluation of the relative amounts of rP6 expressed by the two induced cultures and presence of rP6 in uninduced cultures. The Coomassie stained gel analysis of the uninduced and induced cultures (Declaration Fig. 1A) shows that the induced bacteria containing each plasmid expressed the P6 protein. However, the level of P6 expressed by the BLR(pPX4020) strain is substantially increased relative to the level of P6 expressed by the pPX162 containing strain as shown by the dark rP6 band at ~16 kDa.

As stated in Paragraph 9 of the Green declaration, Western blot analysis was performed to confirm the identity of the induced protein band as the rP6 protein and to detect any expression of the rP6 protein in the uninduced versus induced cultures (Declaration Figure 1B). The amount of protein loaded into the lanes of the gel used for the Western blot analysis was overloaded as compared to the amount used for normal Western blot analysis to increase sensitivity for low amounts of rP6 in the uninduced cultures and thus is not suitable to show differences between the larger amounts of rP6 in the induced cultures. This is due to the amounts of rP6 in these cultures being

outside of the linear range of the Western blot as performed here. The Western blot shown in Figure 1B clearly shows that the rP6 protein is expressed by both pPX162 and pPX4020. Additionally, extremely low levels of rP6 (barely detectable by Western blot) are expressed in the uninduced cultures of the pPX4020 containing strain, as is typical for the tightly regulated arabinose promoter system. In contrast, the pPX162 uninduced culture contains much larger amounts of detectable rP6 protein, demonstrating that the *lac* promoter in the pPX162 is not tightly regulated, allowing significant "leak through" expression in the absence of any inducer.

As stated in Paragraph 10 of the Green declaration, Dr. Green concludes that the level of rP6 protein expressed by plasmid pPX4020 which contains the gene encoding lipidated P6 under control of the arabinose promoter is substantially greater than that expressed by the pPX162 plasmid which contains the same gene under control of the *lac* promoter. Dr. Green's laboratory tried for years to greatly increase expression levels of lipidated P6 protein in *E. coli* and was unsuccessful, as described in their publication from 1990 (Green et al., Infect. Immun. 58(10):3272-3278). Another laboratory also tried and failed to overexpress lipidated P6 protein in *E. coli* and also had to make non-lipidated rP6 many years later (Yang, Y. P., et al., 1997, Vaccine 15:976-87). [Note: Both of these references are cited in the specification at page 3, line 19, and page 4, line 11. They were also included in Applicant's Information Disclosure Statement filed on May 29, 2003 and received by the Patent Office on May 30, 2003.]

As stated in Paragraph 11 of the Green declaration, Dr. Green also concludes from the Western blot analysis of the cultures that the arabinose promoted construct (pPX4020) is tightly regulated and, in the absence of the inducer, expresses levels of rP6 so low that even Western blot can barely detect a band of rP6. This is in direct contrast to the *lac*-promoted rP6 gene contained in plasmid pPX162, which expresses easily detectable amounts of rP6 even in the absence of the IPTG inducer. This is another example of the "leakiness" of the *lac* promoter as described throughout the literature.

Based on the foregoing, Applicant submits that the rejection under 35 USC 112, second paragraph, has been overcome and should therefore be withdrawn.

III. The Rejection Under 35 USC § 102(b)

Claims 1, 2 and 8 remain rejected under 35 USC 102(b) as being anticipated by Anilionis et al. (WO 90/02557) in light of Nelson et al. (*Infection and Immunity*, 56(1):128-134, 1988) for reasons made of record. Applicant traverses the rejection.

Anticipation requires identity of invention. That is, each and every element as set forth in the claim must be disclosed in a single prior art reference, either expressly or inherently. Claim 1 as amended is directed to a plasmid constructed to contain a tightly regulated promoter operatively linked to a gene that encodes a peptidoglycan-associated lipoprotein (recombinant PAL), wherein the recombinant PAL, under the control of the tightly regulated promoter, is expressed in lipidated form and in yields that are higher than those expressed when the recombinant PAL is under the control of a leaky regulated promoter. Anilionis used strong promoters, such as the *lac* promoter, to obtain high levels of transcription, but Anilionis was unable to obtain with such promoters a high level of lipidated P6 expression. Anilionis explicitly discloses in Example 8 (page 81, lines 23-25) that "[w]hen PBOMP-1 was expressed from lac or P_L promoters in *E. coli* JM103 or HB101 strain, only low levels of PBOMP-1 were expressed." Since Anilionis teaches nothing about using a tightly regulated promoter to produce increased levels of PBOMP-1 (i.e., P6) expression, Anilionis does not teach each and every element of the claimed invention. The rejection under § 102(b) is, therefore, improper and should be withdrawn.

IV. The Rejection Under 35 USC § 103(a)

Claims 3-5 remain rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Guzman et al. (*Journal of Bacteriology*, 177(14):4121-4130, 1995) for reasons made of record.

Claims 3 and 6 remain rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Mertens et al. (*Gene*, 164:9-15, 1995) for reasons made of record.

Claims 3, 6 and 7 remain rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Mertens and Novagen Inc. for reasons made of record.

Common to all of these rejections is the Examiner's steadfast reliance on primary reference Anilionis et al. (WO 90/02557). The Examiner argues that Anilionis et al.

teach that for the purpose of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene; and that depending on the host cell system utilized, any one of a number of suitable promoters may be used (page 29, lines 11-16). The Examiner also argues that strong promoters and tightly regulated promoters are not mutually exclusive, i.e., some strong promoters can be tightly regulated. Therefore, she concludes, the suggestion by Anilionis et al. for strong regulatable promoters is met by the secondary references, and hence, Applicant's invention is unpatentable. Applicant disagrees and traverses the rejections.

Applicant first wishes to address the Examiner's assertion that Applicant has again argued the references individually rather than in combination. Applicant has merely grouped the rejections together because Anilionis et al., the primary reference, is common to all of the combinations. In the amendment dated November 12, 2004, Applicant stated: "Consequently, since Anilionis does not teach tightly regulated promoters to improve the expression of lipidated P6, Anilionis does not render the claimed invention obvious. Nor do any of the references combined with Anilionis fill this gap." (Page 7, lines 25-28). In the same amendment, Applicant stated: "Nor does the Examiner's combination of references show or suggest the properties and results of the claimed invention or suggest the claimed combination as a solution for improving the expression of lipidated P6. Consequently, they cannot be successfully relied upon for an obviousness rejection." (Page 9, lines 13-16). See also Applicant's amendment dated February 25, 2004, which discusses establishing obviousness based on a combination of references (page 6, lines 18-19; page 7, lines 22-29 through page 8, lines 1-26).

Turning now to the specific rejections, Applicant still asserts that the Examiner is wrong in including Applicant's tightly regulated promoters under the umbrella of the strong promoters of Anilionis et. If the use of strong promoters were the only consideration, then Anilionis et al.'s use of the *lac* promoter would have resulted in high levels of lipidated P6 expression. It did not. As Applicant has repeatedly pointed out, Example 8 (page 81, lines 23-25) of Anilionis et al. explicitly states that when PBOMP-1 [P6] was expressed from *lac* or P_L promoters in *E. coli*, only low levels of P6 were expressed. See also the Green Declaration included in this Amendment.

As the Examiner states, the name of the game is the claims. One essential element of the claims is Applicant's use of a tightly regulated promoter. Anilionis et al.

do not teach or suggest using tightly regulated promoters, but rather teach the use of strong, leaky promoters. And selecting prior art references that merely contain tightly regulated promoters does not provide the motivation to combine the references to create a case of obviousness.

Guzman et al. constructed vectors (pBAD vectors) containing the tightly regulated P_{BAD} promoter of the arabinose operon and its regulatory gene, *araC*. Guzman et al., however, do not disclose or suggest using this vector system to increase the expression of lipidated P6, another essential element of the claimed invention. At best, Guzman et al. would be viewed by one skilled in the art as an invitation to try their vector system, which is not the standard for determining obviousness.

Mertens et al. constructed a dual-promoter expression plasmid, containing both λ P_L and P_{T7} promoters, for heterologous gene expression in *E. coli*. Using these plasmids, high production levels were obtained for a number of mammalian cytokines (human tumor necrosis factor, human immune interferon, human and murine interleukins 2, murine interleukin 4 and murine fibroblast interferon). Mertens et al. therefore conclude that these plasmids have the *potential* to considerably improve the expression level of other heterologous genes. Again, having the *potential* to improve expression levels does not provide a reasonable expectation of success. It merely invites the skilled artisan to experiment. Mertens et al. even acknowledge that despite the wide experience in the field, high-level expression is often a result of trial and error.

Novagen Inc. supplies the commercially available expression vector pET27b, which has the T7 promoter.

Since neither Anilionis et al. nor any of the secondary references, either alone or in combination, disclose or suggest the claimed invention, they cannot be relied upon for an obviousness rejection. Even if there were motivation to combine the references as the Examiner did, for whatever the reason, none of the Examiner's combinations yield the invention as now claimed. Therefore, Applicant's invention cannot be *prima facie* obvious.

Moreover, the Examiner cannot ignore that other laboratories tried and failed to express usable amounts of lipidated P6, and they all had the same references available to the Examiner to teach them how to do it. (See specification, pages 3 and 4, and the Green Declaration, paragraph 10.) They failed, plain and simple. Applicant figured out

what combination would work, and it is Applicant's plasmid construct that is responsible for increased expressions levels of lipidated P6.

The Examiner states at page 8 of the Office Action that expression in a lipidated form is a property of a vector in a specific host cell and not a structural property of the claimed plasmid. This is incorrect. Expression of the protein in lipidated form is a property of the claimed plasmid because the expressed PAL protein is encoded by a DNA sequence that includes the lipidation signal sequence and processing site. Without this sequence, the host cell would be unable to lipidate the PAL protein.

In view of the foregoing, Applicant respectfully submits that the claims as amended overcome the rejections and, therefore, requests that these rejections be withdrawn.

V. Rejection Under 35 USC §112, first paragraph

Claims 1-8 stand rejected as failing to comply with the written description requirement. Applicant has amended independent claim 1 to overcome this rejection. Support for amended claim 1 can be found, for example, in the specification at page 5, lines 7-11 and lines 16-23; page 7, line 31 through page 9, line 3; and page 3, lines 29-35.

Applicant respectfully requests that a Notice of Allowance be issued in this case.

Respectfully submitted,

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